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BASIC PROTEIN METABOLISM IN RUMINANTS

The University of Nebraska - Lincoln PH.D. 1982

# BASIC PROTEIN METABOLISM IN RUMINANTS

by

Rick A. Stock

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BY

Rick A. Stock

| APPROVED | DATE |
|---|---|
| Terry Klopfenstein | July 29, 1982 |
| Austin Lewis | July 29, 1982 |
| John K. Ward | July 29, 1982 |
| Robert Britton | July 29, 1982 |
| Robert Hill | July 29, 1982 |
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| | |

SUPERVISORY COMMITTEE

GRADUATE COLLEGE UNIVERSITY OF NEBRASKA

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## Introduction

Protein reaching the small intestine of ruminants is primarily of two origins: undegraded protein and microbial protein. The amount of feed protein undegraded in the rumen can be quite variable. Rumen microbial protein synthesis depends on a supply of ammonia, digestible energy and carbon chains for amino acid synthesis. Most of the carbon chains are produced in the degradation of carbohydrates to volatile fatty acids. However, the branched-chain volatile fatty acids may not be produced in sufficient quantities, from low quality roughages, to provide maximum microbial protein synthesis. Some cellulolytic bacteria also require specific amino acids or peptides for maximum growth. Degradation of soluble feed protein can supply these carbon chains, amino acids and peptides. Whey has been used as a source of soluble protein to stimulate nonprotein nitrogen utilization and has given a consistent response in diets low in protein in the basic ingredients.

Effects of low microbial growth rates upon amino acid availability to the animal and upon intake are particularly relevant when low protein roughages, byproduct feeds and nonprotein nitrogen are fed. Low protein roughages and byproduct feeds have a low nitrogen digestibility. When nonprotein nitrogen supplies all the supplemental nitrogen on these diets, microbial growth has been shown to be liting. Feeding a combination of urea and high bypass protein, like blood meal, may also limit microbial growth due to a low dietary supply of amino acids, peptides and branched-chain fatty acids.

Weighing errors cause considerable variation in daily gain in most growth trials. In ruminants these errors are the result of differences in gut fill. When animals are fed in groups, increasing the number of animals per pen will help

reduce weighing variation. However, with pen feeding, a large number of pens, and consequently, animals are needed to test for differences between treatments. With the use of individually fed animals, fewer animals are necessary to test for treatment differences. Only a small amount of research has examined weighing errors and their effect on the variation among individually fed animals.

The research reported herein was conducted to study the value of supplementation of rumen degradable protein and its effect on performance of animals fed low protein diets supplemented with urea, soybean meal or a combination of urea and blood meal. Research was also conducted to study weighing procedures and the variation in nitrogen utilization of blood meal and soybean meal on measured performance of individually fed growing lambs and cattle.

# REVIEW OF LITERATURE

## Microbial Protein Synthesis

Food exposed to bacteria and protozoa in the rumen is digested and fermented for the primary purpose of providing energy and other nutrients for microbial growth (Hungate, 1966). Studies have shown that between 30 and 90% of the flow of nitrogen (N) from the rumen is of microbial origin (Pilgrim et al., 1970; Smith and McAllan, 1970; Mathison and Milligan, 1971). Although microbial protein is a good quality protein with a biological value of 70 to 80% (Lewis, 1970), degradation of high quality protein sources followed by resynthesis into microbial protein represents a protein quality loss to the animal (Dinius, 1976).

Amino acid composition of rumen bacterial protein isolated from sheep on widely different diets has been shown to be similar (Weller, 1957; Purser and Buechler, 1966; Meyer et al., 1967). However, this does not infer that all rumen bacteria are of similar protein quality, since Bergen et al. (1967) demonstrated that 14 strains of rumen bacteria with similar amino acid compositions were of widely differing protein quality.

Studies concerning the quality and importance of protozoa have been contradictory. McNaught et al. (1954) indicated that rumen microbial preparations containing protozoal protein have a higher nutritive quality than those containing only bacterial protein. However, Bergen et al. (1968b) found no differences in protein quality between protozoa and bacteria. Faunated lambs have shown an increase in dry matter digestibility (Klopfenstein et al., 1966) and daily gain (Abou Akkada and El-Shazly, 1964) compared to defaunated lambs

suggesting that protozoa are essential in carbohydrate fermentation in the rumen. The protozoa also play a vital role in slowing down fermentation in the rumen. By ingesting starch grains and taking up soluble sugars and converting them to storage polysaccharides which are relatively slowly metabolized, protozoa greatly reduce the quantities of substrate available to the faster-growing bacteria (Schwartz and Gilchrist, 1975). However, the protozoal population may be detrimental to the animal's utilization of nutrients since they enguf bacteria (Thomas, 1973) and compete for the same substrates within the rumen (Eadie and Hobson, 1962). Weller and Pilgrim (1974) and Bergen and Yokoyama (1977) stated that it was a possibility that there exists within the rumen a cycling protozoa pool with little net protein passage to the lower tract. In this case, ruminal protozoa may actually limit ruminal protein production and depress the supply of amino acids to the host. In theory, it seems plausible that some quantity of protozoa may be needed to maximize turnover rate and improve the efficiency of bacterial growth(Thomas, 1973), while a large quantity of protozoa would decrease this efficiency.

Stern and Hoover (1979) noted several factors that could affect microbial protein synthesis: concentration and source of N and carbohydrates, rumen dilution rate, dietary sulfur and frequency of feeding. Of these, N and carbohydrates have the greatest effect on microbial protein synthesis.

Ammonia is a major source of N for rumen bacteria (Smith, 1975). Bryant and Robinson (1962) found that 82% of freshly isolated strains of bacteria grew well in media containing ammonia and a small amount of cystine as the main source of N. Pilgrim et al. (1970) used $^{15}N$ as a marker to determine the amount of rumen ammonia incorporated into microbial cells. On a low protein diet, 77%

of the bacterial N and 53% of the protozoal N passed through the rumen ammonia pool. These figures were reduced to 63 and 38%, respectively for animals consuming a high protein diet. Nolan et al. (1976) using a similar technique found that only 40% of the N in isolated rumen bacteria was derived from ammonia indicating that a considerable proportion of their N requirement was obtained from compounds other than ammonia (e.g. peptides and amino acids).

Although ammonia has been shown to be used by the rumen microbes, reports have been contradictory on the amount actually needed by the microbes. Several in vitro studies using continuous culture systems have shown maximum microbial growth to occur when the ammonia concentration was 5 to 8 mg/100 ml rumen fluid (Henderson et al., 1969; Satter and Slyter, 1974; Okorie et al., 1977). Satter and Slyter (1974) did note however, that they had found maximum in vivo microbial protein synthesis to occur at 10 to 20 mg $NH_3$/100 ml. Hume et al. (1970) showed with sheep fed semipurified protein-free diets, containing urea as the N source, that increasing the level of urea (2, 4, 9, 16 g N/day) increased ruminal protein production. They concluded that microbial protein synthesis was depressed when rumen ammonia concentration was below 13.3 mg $NH_3$/100 ml of rumen fluid. Mehrez et al. (1977) using the dacron bag technique in situ observed higher levels of ruminal ammonia (23.5 mg $NH_3$/100 ml ) to be optimal for maximum rate of fermentation of barley diets fed to sheep. In contrast, Ortega et al. (1979) found in situ that increasing ruminal ammonia concentrations from 6.3 to 27.5 mg/100 ml did not result in any significant changes in rate of fermentation. The rumen ammonia concentration required for maximal rate of

fermentation does not necessarily equate to maximal protein synthesis (Stern and Hoover, 1979).

Feeding diets that produce inadequate rumen ammonia levels for microbial growth will decrease quantity of the fiber digested. Campling (1962) reported that when urea was added to oat straw diets consumed by cows, intake and organic matter digestion were increased. Addition of urea to lowly degradable protein diets has been shown to improve in vitro rumen cellulose digestion and increase feed consumption and daily gain of lambs (Little et al., 1963) as well as increase dry matter digestibility with lambs (Klopfenstein et al., 1976). Krause and Klopfenstein (1978) demonstrated a complimentary effect of feeding dehydrated alfalfa and urea to steers. These results demonstrate the effects of N limiting microbial growth and depressing fiber digestion.

Microbial protein synthesis can occur in the rumen on diets in which urea is the only N source, however, efficiency of microbial growth may be limited by a deficiency of preformed amino acids or peptides. Although the concentration of free amino acids in the rumen is low and they are rapidly metabolized (Wright and Hungate, 1967ab), Maeng et al. (1976) using a batch culture system found a ratio of 3:1 for urea N:amino acid N optimal for bacterial growth. Peptides are used by and reportedly stimulate growth of both pure and mixed cultures (Pitman and Bryant, 1964; Wright, 1967). The identification of specific amino acids or peptides limiting microbial growth may depend on the diet fed. Hume (1970) found that N provided from urea, gelatin, casein and zein resulted in microbial synthesis of 17.1, 19.8, 23.3 and 22.5 g CP/100 g organic matter digested in the rumen of sheep. Since degradation of gelatin and casein in the rumen approached completion, microbial protein production on the gelatin diet may have been

limited by the rate of synthesis of one or more amino acids by the rumen bacteria, particularly methionine. Only 13% of the zein, which is relatively high in methionine, was degraded in the rumen. Therefore, the amino acid requirements of the rumen bacteria must be quite specific in view of the response to the small amount of zein degraded compared to gelatin. Salter et al. (1979) found that when an adequate dietary supply of preformed amino acids were fed to steers, proline, arginine, histidine, methionine and phenylalanine were synthesized to a greater extent from preformed units than other amino acids. While synthesis of proline, arginine, and histidine increased when steers were fed a urea diet, methionine and phenylalanine did not. Therefore, methionine and phenylalanine may be limiting for bacterial growth on diets low in protein and high in urea. Potter et al. (1964) and Amos et al. (1971) demonstrated consistent increases in cellulose digestion in vitro with distillers solubles supplementation. Because distillers dried solubles contain large amounts of proline which is converted to valeric acid by rumen microbes, branched-chain amino acids may be required to support maximum microbial growth. Teather et al. (1980) reported that diets of corn silage and concentrate, when supplemented with soybean meal or corn silage with urea added at the time of ensiling, supported a ruminal bacterial population 70% greater than diets supplemented with urea added to the concentrate. The greatest increases were observed among bacterial species known to require branched-chain fatty acids for optimum growth. They suggested that dietary amino acids were degraded to form volatile fatty acids to a greater extent rather than incorporated directly into microbial protein.

Growing calves fed corn silage diets supplemented with a combination of

urea and dried whey gained faster and more efficiently than calves supplemented with urea alone (Hendrix, 1974). Cook et al. (1982) showed a similar effect feeding alfalfa with chemically treated corn cobs and a 50:50 urea:bypass protein supplement. The authors noted this resopnse could be due to chemical dilution, supplementation of required minerals, slowed passage from the rumen or stimulation of microbial growth.

Many of the amino acids limiting bacterial growth may also be limiting in bacterial protein synthesized. Bergen et al. (1967) using an in vitro enzymatic degradation system, found methionine and leucine to be the first limiting amino acids in cellulolytic and non-cellulolytic strains of bacteria, respectively. Later Berger et al. (1968a) using a plasma amino acid score, reported that the order of limiting amino acids was cystine, arginine, histidine, leucine and lysine. Nimrick et al. (1970) determined the limiting amino acids of rumen synthesized protein by infusing individual amino acids into the abomasum of growing lambs fed semipurified diets. The suggested order of limiting amino acids was methionine, lysine and threonine. This is in agreement with work by Richardson and Hatfield (1978) who also determined that methionine, lysine and threonine were the first three limiting amino acids in bacterial protein synthesized on steer diets. Leibholz (1972) using sheep fed semipurified diets determined methionine flow to the duodenum was sufficeint to meet the maintenance requirement of sheep, but it would not be sufficient to meet the sulphur amino acid requirement for maximum wool growth. Thus, the source of supplemental N and the extent of degradability of the N source play a major role in determining the quantity and quality of microbial protein produced.

Efficient utilization of degraded dietary N requires that the energy from the fermentation of dietary organic matter must be supplied at a rate which matches the synthetic abilities of the rumen microbes (Oldham et al., 1977). Addition of readily available carbohydrates such as sugars and starches have been shown to increase microbial growth and/or increase utilization of dietary true protein. Stern et al. (1978) using a continuous flow fermentation system determined that replacing cellulose with starch resulted in decreased fiber digestion, decreased ammonia levels and increased microbial protein synthesis. Amos et al. (1979) using abomasally fitted wethers observed an increase in microbial protein synthesis from diets containing a combination of corn starch and formaldehyde treated bermuda grass hay compared to diets containing solka flow (powdered cellulose) and formaldehyde-treated bermuda grass hay. Kennedy (1980) demonstrated that sucrose additions to steer diets improved the efficeincy of rumen ammonia incorporation into microbial protein. Addition of readily available carbohydrates may stimulate cellulose digestion at levels of 5 to 10% (Burroughs et al., 1950; Chapell and Fontenot, 1968) but may decrease cellulose digestion at higher (20%) levels (Amos and Evans, 1980). Thus, the major factors affecting the utilization of degraded dietary N are the rate of availability of carbohydrates (Stern et al., 1978) and the amount of carbohydrates fed (Amos and Evans, 1980).

Volatile fatty acids with branched and straight chains of four and five carbons are essential for growth of rumen cellulolytic microorganisms (Allison et al., 1962). In vitro studies (Beeson and Perry, 1952; Bentley et al., 1955) have shown that valeric and isovaleric acids increase cellulose digestion. Hungate and Dyer (1956) increased intake and growth rate of steers fed straw diets by

inclusion of isoacids into their diets. This is in agreement with the results of Lassiter et al. (1958), who found an increase in gain of dairy heifers fed valeric and isovaleric acids. Feeding isovaleric and/or isobutyric acids to lambs on high roughage, urea supplemented diets improved N retention and decreased urinary N loss (Umunna et al., 1975). Addition of a mixture of isobutyrate, isovalerate, 2-methylbutyrate, valerate and phenylacetate to high roughage diets fed to dairy heifers increased the growth rate of younger animals (200 kg bodyweight) but not older ones (285 kg bodyweight) (Felix et al., 1980b). The authors believed this was due to a greater protein acretion of the younger animals than the older animals. These authors also showed that the isoacid mixtures decreased urinary N and increased N retained by lactating dairy cows. Felix et al. (1980a) also showed that lactating dairy cows fed corn silage diets had improved milk production and persistency of lactation when a combination of urea and isoacids were fed compared to urea alone. In order to supply these branched-chain fatty acids, diets do not have to be supplemented with purified isoacids, because degradation of soluble feed proteins can supply these carbon chains (Oltjen et al., 1971; Klopfenstein, 1980).

Dilution rate has been shown to be positively correlated with increased microbial growth (Hobson and Summers, 1967; Isaacson et al., 1975; Harrison et al., 1976). Several factors have been found to alter dilution rate. Increasing frequency of feeding (Hungate et al., 1971), increasing dry matter intake (Singh et al., 1977) and decreasing environmental temperature (Kennedy and Milligan, 1978) increased both dilution rate and microbial synthesis. Cole et al. (1976) observed increases in rumen dilution rate and protein synthesis when steers were switched from an all-concentrate diet to one containing 86% concentrate. Allen

and Harrison (1979) found that feeding rumensin to sheep decreased rumen dilution rate and decreased microbial synthesis.

Kennedy et al. (1976) suggested that a combination of factors may be involved in causing microbial synthesis to be positively correlated to dilution rate: reduced autolysis of bacteria; reduced engulfment of bacteria by protozoa; changes in microbial population structure induced by a change in substrate or possibly due to washout of slow generation time organisms.

## Methods of Estimating Microbial Protein Synthesis

Various methods have been used to estimate the quantity of microbial protein leaving the rumen. The majority of these techniques utilize a marker and are based on the assumption that the marker characterizes only the microbial component. All marker methods have several inherent problems. These problems include presence of the marker in feed material, measurement of the marker in a representative rumen microbial population and extensive analytical techniques to measure the marker. Several marker techniques have been proposed. Diaminopimelic acid (DAP), aminoethylphosphonic acid (AEP), nucleic acids and isotopes ($^{35}S$, $^{15}N$, $^{32}P$) incorporated into proteins in the rumen have been used for this purpose. Recently D-alanine, amino acid profiles of individual components and adenosine triphosphate (ATP) have been proposed as markers for microbial protein synthesis.

DAP is a cell wall constituent of several bacteria and has been widely used to estimate bacterial protein synthesis. The potential limitatoins in using DAP appear to have received more attention in the literature than other marker systems, probably because its use predates the others. Assumptions inherent in the use of DAP as a marker for bacterial N are that it is absent from other

nitrogenous components in the rumen, omasum and abomasum, and that the DAP:N ratio determined for the sample of rumen bacteria is truly representative of the total population of bacteria in the rumen.

DAP reportedly is absent in protozoa and plant material (Ibrahim et al., 1970); however, due to the engulfment of bacteria (Coleman and Hall, 1969; Thomas, 1973; Stern et al., 1977ab), the presence of small quantites of DAP in protozoa has been reported (Weller et al., 1958; Hutton et al., 1971; Ling and Buttery, 1978). Theurer (1982) reported levels of DAP-N in feeds (expressed as a percent of total N in the sample) ranging from 12 to 78% of reported DAP concentrations in bacteria. He also noted that the Handbook of Biochemistry (1970) indicates that a source of DAP is pine pollen and thus would be expected to be contained in some feeds. This work is in contrast to the results of Ibrahim et al. (1970), Hutton et al. (1971) and Ling and Buttery (1978) who reported that DAP was not detected in dietary constituents. Theurer (1982) noted that the higher levels of feed DAP noted in his studies may in part be due to the elution of unknown acids in the "DAP peak" thus causing overestimation of the DAP. Thus, it is important to use analytical techniques which allow DAP to elute alone to prevent incorrect conclusions of DAP concentrations in protozoa and feed sources.

The accuracy of the DAP method is also dependent on a constant DAP:N ratio among various microbial species or the maintenance of a constant ratio of microbial species in the rumen. Despite the fact that the DAP:N ratio has been shown to vary among species (Work and Dewey, 1953; Purser and Beuchler, 1966), Weller et al. (1958) and Hutton et al. (1971) showed that the DAP:N ratio for mixed rumen bacterial samples from sheep on a fixed feeding regime is

reasonably constant.

AEP was suggested as a marker for protozoa by Abou Akkada et al. (1968). They detected no AEP in washed suspensions of mixed rumen bacteria or in pure strains of Streptoccus bovis, Selenomonas ruminatium or Bacteriodes succinogenes. The diet was also devoid of AEP. The major part of AEP in rumen contents was found to be bound to the solids. The small amount in the free state was probably due to ruptured protozoal cells. Borhami et al. (1979) reported that the mean microbial N incorporation in the rumen contents using DAP and AEP as markers for bacterial and protozoal N was similar to the value obtained from $^{15}N$ incorporation in microbial cells. In constrast, Ling and Buttery (1978) and Theurer (1982) considered AEP an unsatisfactory marker of protozoa. Ling and Buttery (1978) noted AEP in samples of protozoa, bacteria and dietary constituents. Theurer (1982), using automated amino acid analysis, reported no AEP in feed, mixed rumen protozoa or abomasal samples. Recently, Dufva et al. (1982) found bacteria and different feeds to be devoid of AEP. They noted a high correlation (r=.84) between AEP-N:total-N ratio and lipid content of protozoa. They concluded that to use AEP, it would be necessary to correct for dietary effects based on the species composition of rumen protozoa and on their chemical composition.

Interest recently has been centered around nucleic acids as markers for microbial protein synthesis. Results using total nucleic acids (ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)) or RNA and DNA alone have been reported. Work conducted with purines and pyrimidines in the polynucleotide form are also currently being investigated. Smith (1969) reported that in samples of rumen fluid taken from calves 4 h after feeding a variety of diets, 10 to 16%

of their total nonammonia N was in the form of nucleic acids. Similar conclusions have also been reported by Smith et al. (1968) and Smith and McAllan (1970), who have suggested that total nucleic acids are a relatively constant indicator of total microbial protein synthesis. Other researchers have demonstrated variations related to rate and stage of microbial growth. Adams et al. (1976) reported that the nucleic acids in mature bacteria can account for 10 to 15% of the cell dry-weight, while the values range as high as 21% for rapidly growing bacteria. It is the concentration of DNA in the cell that is believed to be responsible for this variation in nucleic acid content (Smith, 1969) since the DNA content of a cell remains constant while the cellular protein content can change considerably depending upon its physiological stage (Schelling et al., 1982).

The use of nucleic acids as a marker depends on the assumption that the nucleic acids isolated from a duodenal sample are of microbial origin. Pure RNA and DNA infused into the rumen have been shown to be rapidly and almost totally degraded within the rumen (Smith and McAllan, 1970). However, since significant amounts of some dietary materials often escape degradation in the rumen, it is not unlikely that the nucleic acids associated with these undegraded dietary fractions also contribute to the duodenal digesta nucleic acids (Ling and Buttery, 1978). Another disadvantage of nucleic acids is the low estimation of a protozoal fraction in digesta. Although bacteria usually account for the greater proportion of the microbial N reaching the duodenum, the contribution of protozoal N should not be neglected. Ratios of nucleic-N:total-N (Smith and McAllan, 1970) or RNA-N:total-N (Ling and Buttery, 1978) of protozoa have been shown to be lower than those of bacteria. Where the contribution of protozoal N

is large, underestimation of duodenal microbial N would occur. McAllan and Smith (1974) reported larger microbial N:total nonammonia N ratios in duodenal contents using RNA compared to DAP. Unless considerable dietary RNA survived rumen degradation, RNA estimated more protozoal N than their DAP method. In view of the variations observed in RNA:DNA ratio and the fact that RNA shows less variation relative to total-N in different microbes than either DNA or total nucleic acid (Smith, 1969), RNA alone may be a better index of microbial N than either DNA or total nucleic acids (Smith and McAllan, 1970).

In an effort to develop a more satisfactory analytical procedure, work has been conducted using the purine and pyrimidine bases as indicators of microbial protein synthesis. Koenig et al. (1980) reported that cytosine, adenine, guanine, uracil and xanthine are acceptable indicators of microbial protein synthesis. Cytosine and adenine were considered to be the bases with the greatest potential as indicators. Zinn and Owens (1982) reported a rapid assay for purine content of feed and digesta utilizing a silver nitrate precipitation procedure. More work is needed to test the validity of these two techniques.

Of the radioisotopes used as tracers to distinguish between microbial and dietary protein, $^{35}S$ has been used most frequently. Walker and Nader (1968) used radioactive sodium sulfide to label the sulfide pool rumen digesta in vitro. The principle of that method was that all sulfur incorporated into microbial protein was first passed through the free $H_2S$ pool rather than direct incorporation of preformed sulfur amino acids. While this may be true in vitro, in vivo studies have revealed that considerable amounts of sulfur containing amino acids in microbial protein may arise from sulfur which has not passed through the $H_2S$ pool (Gawthorne and Nader, 1976; McMeniman et al., 1976;